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A Method for Aquatic Multiple Species Toxicant Testing: Acute Toxicity of 10 Chemicals to 5 Vertebrates and 2 Invertebrates

Gary L. Phipps & Gary W. Holcombe

US Environmental Protection Agency, Environmental Research Laboratory-Duluth, 6201 Congdon Boulevard, Duluth, Minnesota 55804, USA

ABSTRACT

A method was developed to simultaneously ascertain 96 h LC_{50} values for seven freshwater species in a single flow through test with measured concentrations. It allows interspecific comparisons, easy determination of the most sensitive species, and cuts cost of labour, materials and chemical analysis for measured concentration tests. Species tested included fathead minnows Pimephales promelas, *goldfish* Carassius auratus, *channel catfish* Ictalurus punctatus, *bluegill* Lepomis macrochirus, *rainbow trout* Salmo gairdneri, *crayfish* Orconectes immunis *and snails* Aplexa hypnorum. *Compounds tested were pentachlorophenol, 2-chloroethanol, 2,4-pentanedione, hexachloroethane, α-bromo-2′,5′-dimethoxyacetophenone, benzaldehyde, 1,3-dichloro-4,6-dinitrobenzene, dursban, sevin and cadmium chloride. The LC_{50} values from these multiple species tests compared favourably with those determined using single species tests at this laboratory, usually within 20%.*

INTRODUCTION

The objective of this study was to develop a more efficient method for conducting aquatic acute toxicity tests so the resulting data would be directly comparable between species. This method produced LC_{50} data on several species at a fraction of the time and money it takes to produce them by using individual tests for each species. To protect the aquatic

environment from pollution by a particular chemical a variety of organisms must be tested. The Water Quality Criteria Guidelines (USEPA, 1980) requires data on at least eight freshwater families before criteria for the protection of freshwater life can be promulgated. This method produces data that satisfies five of those eight requirements by testing five vertebrates and two invertebrates simultaneously.

Species tested included fathead minnows *Pimephales promelas*, goldfish *Carassius auratus*, channel catfish *Ictalurus punctatus*, bluegill *Lepomis macrochirus*, rainbow trout *Salmo gairdneri*, crayfish *Orconectes immunis* and snails *Aplexa hypnorum*. In addition, chinook salmon *Oncorhynchus tshawytscha* were tested with the pesticide sevin. Rainbow trout and crayfish were not initially tested with pentachlorophenol. The compounds selected for testing were pentachlorophenol (PCP), 2-chloroethanol, 2,4-pentanedione, hexachloroethane, α-bromo-2′,5′-dimethoxyacetophenone, benzaldehyde, 1,3-dichloro-4,6-dinitrobenzene, dursban, sevin and cadmium chloride.

A method of conducting these comparative toxicity tests was developed that entailed testing all species in separate screened compartments of the same exposure tank. To verify this method, tests with PCP were conducted in duplicate where each species was tested in individual exposure tanks (the traditional method) and then in different compartments of the same exposure tanks (the newly developed method) so comparisons could be made between methods. Tests were conducted at 17 °C, since this is one of the recommended test temperatures for warm water organisms (ASTM E729, 1980) and 16–18 °C is the optimum temperature for rainbow trout based on measures of physiological performance and activity (Lichtenheld, 1966; Lavrovsky, 1968; Dickson & Kramer, 1971; McCauley & Pond, 1971; Hokanson *et al.*, 1977).

Reasons for conducting comparative toxicity tests include: (1) to find a chemical of high toxicity to a pest and low toxicity to nontarget organisms; (2) to determine the most sensitive organisms so they may be environmentally protected; (3) to determine sensitivity differences between various species and establish if and how sensitivity differences change with various compounds or groups of compounds that exhibit different modes of toxic action; and (4) to assess how many different species need to be tested in order to set adequate water quality criteria to protect the environment. Appelgate *et al.* (1957) conducted tests with two species of fish and sea lamprey *Petromyzon marinus* to find a lampricide, which is an example of the first major reason mentioned above. Data cited

by Pickering & Henderson (1966), Holcombe *et al.* (1982) and in the present paper are examples of the latter reasons.

The US Environmental Protection Agency neither recommends nor endorses any commercial product; trade names are used only for identification.

MATERIALS AND METHODS

Exposure system

Two modified nearly identical 2-litre proportional diluters (Mount & Brungs, 1967) with dilution factors of 0·6 were used for all tests. During each diluter cycle, flow-splitting chambers (Benoit & Puglisi, 1973) divided each of five exposure concentrations and a control into duplicate exposure tanks. Exposure tanks in one system were glass aquaria that measured 50·0 × 25·0 × 30·0 cm deep with an 18·0 cm standpipe that produced a tank volume of 22·5 litres. Each tank was divided into six 16·6 × 12·5 cm wide compartments using stainless steel screen dividers. Glass aquaria in the other system measured 60·0 × 30·0 × 30·0 cm deep with a 17·0 cm standpipe and a tank volume of 30·6 litres. All tanks were divided into six 16·6 m × 15·0 compartments and one 10·0 × 30·0 cm wide compartment with stainless steel screen dividers. The only major difference between the two systems was tank size. This difference was determined by availability and not for any specific scientific purpose and two systems were used to speed testing. Flow rates and 90% replacement times (Sprague, 1969) were 35 ml min^{-1} and 8 h in the first two PCP tests; 67 ml min^{-1} and 13 h in the third PCP test; 130 ml min^{-1} and 8 h in the dursban and one cadmium test; 200 ml min^{-1} and 5·8 h in the sevin test; 263 ml min^{-1} and 3 h in the cadmium test with fathead minnows and goldfish; and 130 ml min^{-1} and 6 h during tests with all other compounds. Flow rates were varied with the size of exposure tanks and according to the size of exposed fish to maintain adequate (> 50% of saturation) dissolved oxygen levels. A 16-h photoperiod was used. Fluorescent lamps provided a light intensity of 28 lumens at the water surface.

Water characteristics

All test water was obtained from Lake Superior and heated to the appropriate test temperature. Means and standard deviations of test temperatures were 22·5 ± 0·4 °C for the PCP tests and 17·3 ± 0·6 °C

during all other tests. Dissolved oxygen (DO) was measured using a dissolved oxygen meter (calibrated using the azide modification of the Winkler method on a minimum of one complete set of duplicate tanks (6) and in most cases on all tanks once during each test. The means, standard deviations and ranges of DO measurements (mg litre^{-1}) were 6·7 ± 1·4 (4·0–8·4) $N = 60$ during the three PCP tests and 7·5 ± 1·6 (4·7–10·0) $N = 148$ during all other tests. The DO concentrations were never less than 50% and seldom less than 60% during all tests. All other water characteristics were measured using methods described by the American Public Health Association *et al.* (1980). During each test, hardness, alkalinity and pH determinations were made, at a minimum, on a control, one intermediate, and one high concentration tank. The means and ranges for total hardness and alkalinity measurements were 44·4 (40·7–46·6) and 45·4 (42·3–57·0) mg litre^{-1} as $CaCO_3$, respectively, for all tests. The pH range during all tests was 7·1 to 7·8.

Toxicant solutions

Dursban and pentachlorophenol were obtained from Dow Chemical Company (Agricultural Product Department, Midland, MI 48640); cadmium was obtained from Matheson, Coleman and Bell (Norwood, OH 45212); sevin was obtained from Union Carbide Corporation (Research Triangle Park, NC 27709); and all other tested chemicals were obtained from Aldrich Chemical Company, Inc. (Milwaukee, WI 53233). Pentachlorophenol, 2-chloroethanol, 2,4-pentanedione, benzaldehyde and cadmium were dissolved in Lake Superior water and a predetermined portion of each solution was introduced to the diluter during each diluter cycle. Due to their low water solubility, all other tested chemicals were dissolved in dimethylformamide (DMF) and a small amount of this DMF stock solution was metered into the diluter during each cycle. Nominal concentrations of DMF in the high test tanks were 108 mg litre^{-1} during all tests with α-bromo-2′,5′-dimethoxyacetophenone, 1,3-dichloro-4,6-dinitrobenzene and dursban; 327 mg litre^{-1} with hexachloroethane, and 477 mg litre^{-1} with sevin.

Water concentrations were analysed four times during all 96-h exposures. Daily water samples for chemical analysis were taken from one complete set of duplicate tanks in the dursban crayfish test, the cadmium rainbow trout test and in all exposures with sevin. Daily water samples were taken from all tanks in the remaining tests.

Chemical analyses for pentachlorophenol (PCP); α-bromo-2′-5′-dimethoxyacetophenone; 1,3-dichloro-4,6-dinitrobenzene and hexachloroethane were performed with a Hewlett-Packard 5730A gas chromatograph (GC) equipped with a Model 3552A data system and a ^{63}Ni electron capture detector. Prior to GC analyses, 1,3-dichloro-4,6-dinitrobenzene samples were extracted with a 75% hexane 25% ether mixture; α-bromo-2′,5′-dimethoxyacetophenone, hexachloroethane, and PCP were extracted with hexane; and the PCP hexane extract derivatised using *N*-ethyl-*N*′-nitro-*N*-nitrosoquanidine. Analyses for dursban were conducted on a Hewlett-Packard 5710A gas chromatograph with an autosampler and a ^{63}Ni electron capture cell. Water samples for dursban were also extracted with hexane before GC analysis. Analyses for 2-chloroethanol, 2,4-pentanedione and benzaldehyde were performed using direct aqueous injection on a Hewlett-Packard 5730A gas chromatograph equipped with dual flame ionisation detectors. Water samples containing cadmium were acidified to 0·5% with concentrated nitric acid and analysed by atomic absorption spectrophotometry. A Perkin-Elmer 5000 spectrophotometer equipped with an HGA graphite furnace was used for samples in the micrograms per litre concentration range, while a Perkin-Elmer 403 equipped with an air–acetylene flame was used for samples in the mg litre^{-1} range. A quality assurance sample from the EPA Environmental Monitoring and Support Laboratory in Cincinnati, Ohio, was analysed twice giving values of 1·44 and 1·51 μg litre^{-1} (true value = 1·50 μg litre^{-1}). Water samples taken during the sevin exposures were passed through a 0·45 micron Millipore filter to remove particles and analysed using a Hewlett-Packard model 1084B liquid chromatograph equipped with a variable wavelength detector and autosampler. Concentrations of all tested chemicals were determined by comparison to a standard curve of known concentrations. Measured water concentrations and percentage spike recoveries for each test for all chemicals are presented in Table 1. Final concentrations were not corrected for recovery with the exception of α-bromo-2′,5′-dimethoxyacetophenone which had a high percent recovery.

Biological procedures

Fathead minnows and snails were obtained from stock-culture units of the Environmental Research Laboratory-Duluth. Minnows and snails were kept in a rearing system at 20 °C and were fed live brine shrimp until

TABLE 1

Measured Water Concentrations (mg litre $^{-1}$) and Percentage Spike Recoveries for the Comparative Toxicity Tests Conducted with All Compounds

Compound	*Tank No. 1 (Control)*	*Tank No. 2*	*Tank No. 3*	*Tank No. 4*	*Tank No. 5*	*Tank No. 6*	*Percentage spike recoveries*
Pentachlorophenol	—[a]	0·059 ± 0·010[b]	0·105 ± 0·013	0·160 ± 0·030	0·288 ± 0·030	0·492 ± 0·035	94·5 ± 11·6
Pentachlorophenol	—	0·040 ± 0·005	0·066 ± 0·007	0·103 ± 0·016	0·179 ± 0·029	0·313 ± 0·038	95·0 ± 5·0
Pentachlorophenol	—	0·031 ± 0·003	0·060 ± 0·007	0·096 ± 0·010	0·181 ± 0·011	0·347 ± 0·009	100·6 ± 4·0
2-Chloroethanol	—	10·0 ± 2·7	16·1 ± 1·5	25·5 ± 3·9	45·9 ± 4·7	80·9 ± 3·7	100·8 ± 3·0
2,4-Pentanedione	—	29·6 ± 2·0	43·5 ± 1·4	62·8 ± 2·4	111 ± 5	217 ± 12	88·9 ± 3·8
Hexachloroethane	—	0·290 ± 0·050	0·410 ± 0·060	0·730 ± 0·010	1·28 ± 0·17	2·10 ± 0·39	92·8 ± 2·6
α-Bromo-2′,5′-dimethoxyacetophenone	—	0·018 ± 0·001	0·027 ± 0·001	0·036 ± 0·002	0·092 ± 0·008	0·227 ± 0·025	134·0 ± 5·9[c]
Benzaldehyde	—	1·44 ± 0·41	2·82 ± 0·42	4·10 ± 0·46	7·96 ± 1·36	15·8 ± 2·1	96·9 ± 2·1
Benzaldehyde	—	[d]	0·130 ± 0·010	0·210 ± 0·010	0·440 ± 0·140	0·800 ± 0·210	98·0 ± 2·0
1,3-Dichloro-4,6-dinitrobenzene	—	[d]	[d]	0·016 ± 0·004	0·044 ± 0·013	0·126 ± 0·012	94·0 ± 5·1
Dursban	—	0·004 ± 0·000	0·007 ± 0·001	0·010 ± 0·001	[d]	[d]	99·8 ± 1·7
Dursban	—	0·171 ± 0·024	0·264 ± 0·023	0·267 ± 0·032	0·433 ± 0·035	0·806 ± 0·094	102·0 ± 3·0
Dursban	—	0·002 ± 0·000	0·004 ± 0·000	0·004 ± 0·000	0·010 ± 0·002	0·023 ± 0·005	101·0 ± 3·0
Cadmium	—	0·313 ± 0·024	0·617 ± 0·036	0·906 ± 0·028	1·86 ± 0·06	3·26 ± 0·05	95·5 ± 3·0
Cadmium	—	[d]	2·35 ± 0·12	3·23 ± 0·25	6·21 ± 0·35	10·2 ± 0·1	103·7 ± 9·2
Cadmium	—	0·001 ± 0·000	0·001 ± 0·000	0·001 ± 0·000	0·002 ± 0·000	0·004 ± 0·000	107·0 ± 4·0
Sevin	—	0·859 ± 0·097	1·66 ± 0·14	2·25 ± 0·43	3·87 ± 0·26	6·52 ± 0·40	92·2 ± 1·3
Sevin	—	3·27 ± 0·43	6·46 ± 0·21	10·2 ± 1·1	15·1 ± 2·0	26·0 ± 1·3	92·2 ± 2·8

[a] Concentrations in the controls were not detectable.

[b] Mean and standard deviation.

[c] Concentrations of α-bromo-2′,5′-dimethoxyacetophenone were corrected for percentage spike recovery.

[d] Concentrations were not measured since no fish were exposed in these tanks.

used for testing. Juvenile rainbow trout, bluegills, and adult crayfish were obtained from Fattig Hatcheries, Brady, Nebraska. Goldfish were obtained from Grassyforks Fisheries Company, Martinsville, Indiana, and channel catfish were obtained from Senecaville National Fish Hatchery, Senecaville, Ohio. Trout and crayfish were held in 530-litre fibreglass holding tanks at 15 °C and fed a commercial dry food. Bluegills, goldfish and catfish were held in 530-litre fibreglass tanks at 17 °C. Bluegills were fed frozen adult brine shrimp and goldfish and catfish were fed a commercial dry food. All species were not fed for 24 h before or during testing.

The number of organisms exposed in each tank and the life stage or size of the organisms used during each test are given in Table 2. Mean weights of the test organisms were determined by weighing the control group at the end of the 96-h exposures. All fish species and crayfish were counted into individual compartments of the 12 exposure tanks. Compartments containing rainbow trout and crayfish were covered with a stainless-steel screen to prevent the test organisms from escaping. Snails were counted into 10 × 3 cm diameter stainless-steel screen cylinders which were closed on one end by a stainless-steel screen and on the other end by a No. 7 neoprene stopper. The cylinders were then randomly distributed to a selected compartment of each exposure tank. Death was the primary endpoint for all species, although effects on equilibrium, behaviour, coloration and deformities were noted. Determinations of LC_{50} values were made using a computerised Trimmed Spearman-Karber method (Hamilton *et al.*, 1977). Mortality data from the duplicate tanks were combined before LC_{50} determinations were conducted. Other procedures or methods not specified followed those recommended by the American Society for Testing and Materials (1980).

Three pentachlorophenol tests (PCP) were conducted using fathead minnows, goldfish, bluegills, channel catfish and snails to determine if testing all species in screened compartments in the same tank was a valid approach. Test 1 tested each species in separate tanks in the same system. The system had a series of 5 PCP concentrations and a control in 4 separate series for a total of 24 exposure tanks. The diluter was the same as the one previously described except each test concentration was split four ways before delivery to the test chambers. Test 2 was a duplicate of test 1 so the variability in the test method could be determined. Test 3 was essentially the same as tests 1 and 2 except, rather than testing species individually, all the species were combined in different screened

TABLE 2

Mean Weight in Grams or Life Stage and the Number of Organisms Exposed Per Concentration (in parentheses) in the Various Comparative Toxicity Tests

Compound	*Diluter flow rate* ($ml\,min^{-1}$)	*Fathead minnows*	*Rainbow trout*	*Goldfish*	*Channel catfish*	*Bluegill*	*Chinook salmon*	*Crayfish*	*Snails*
Pentachlorophenol	35	0·5[a] (10)	—[b]	1·9 (10)	1·4 (10)	0·2 (10)	—[b]	—[b]	Adults (10)
Pentachlorophenol	35	0·4 (10)	—[b]	1·2 (10)	1·4 (10)	0·3 (10)	—[b]	—[b]	—[b]
Pentachlorophenol	67	0·4 (20)	—[b]	1·7 (20)	1·9 (20)	0·4 (20)	—[b]	—[b]	Adults (20)
2-Chloroethanol	130	0·4 (20)	2·2 (20)	2·9 (20)	3·2 (20)	0·4 (20)	—[b]	2·0 (10)	Adults (20)
2,4-Pentanedione	130	0·4 (20)	0·7 (20)	4·3 (20)	4·1 (20)	0·6 (20)	—[b]	2·0 (10)	Adults (20)
Hexachloroethane	130	0·3 (20)	1·8 (20)	8·4 (20)	5·6 (20)	0·7 (20)	—[b]	2·2 (10)	Adults (20)
α-Bromo-2′,5′-dimethoxyacetophenone	130	0·3 (20)	1·7 (20)	6·2 (20)	4·6 (20)	0·7 (20)	—[b]	1·8 (10)	Adults (10)
Benzaldehyde	130	0·8 (20)	2·4 (20)	5·7 (20)	4·6 (20)	0·8 (20)	—[b]	1·8 (10)	Adults (10)
1,3-Dichloro-4,6-dinitrobenzene	130	0·3 (20)	2·0 (20)	7·6 (20)	5·7 (20)	0·7 (20)	—[b]	2·0 (10)	Adults (10)
Dursban	130	0·5 (20)	3·0 (20)	10·7 (20)	7·9 (20)	0·8 (20)	—[b]	1·8 (10)	Adults (10)
Cadmium	130–263	0·6 (20)	8·8 (20)	8·8 (20)	7·4 (20)	1·0 (20)	—[b]	1·8 (10)	Adults (10)
Sevin	200	0·3 (20)	19·7 (20)	14·2 (20)	27·6 (20)	0·5 (20)	3·0 (20)	3·9[c] (3)	Adults (20)
Dimethylformamide (DMF)	200	0·3 (40)	2·1 (20)	19·4 (20)	38·8[d] (4)	2·1 (20)	6·6 (20)	—[b]	Adults (20)

[a] Mean weights were determined by weighing a minimum of 10 organisms exposed in the control tanks.
[b] This species was not tested during this exposure.
[c] Mean weight of 3 organisms.
[d] Mean weight of 4 organisms.

compartments of the same test tank. This means that all species were exposed to the same water in the same tank.

RESULTS

Pentachlorophenol tests

The method of exposing all species in separate compartments of the same exposure tank was validated with results from three pentachlorophenol tests (Table 3) with four species of fish and one species of snail. When the results of pentachlorophenol tests 1 and 2, which were repeat tests using standard methods described by US Environmental Protection Agency Committee on Methods for Toxicity tests with Aquatic Organisms (1975), are compared with the results of test 3, the newly developed method, the results were quite comparable. The 96 h LC_{50} for fatheads and catfish fell within the range of those produced in tests 1 and 2 for these species (Table 3). Goldfish were slightly less sensitive in test 3 (96 h LC_{50} of 0·191 mg litre^{-1}) than in tests 1 and 2 (96 h LC_{50} values of 0·117 and 0·156 mg litre^{-1} respectively), although the difference between tests 3 and 2 was less than the difference between tests 2 and 1. Bluegills were slightly more sensitive in test 3 with a 96 h LC_{50} of 0·115 mg litre^{-1} while tests 1 and 2 gave 96 h LC_{50} values of 0·150 and 0·152 mg litre^{-1} respectively. Snail 96 h LC_{50} values were 0·157 mg litre^{-1} in test 1 and 0·142 mg litre^{-1} in test 3.

Comparative toxicity tests

The 96 h LC_{50} values, their 95% confidence limits and the fish sensitivity factors for the seven tested species with ten chemicals are given in Table 3. Of the five fish species tested, goldfish were least sensitive with five chemicals, while fatheads and bluegills were least sensitive three and two times respectively. Rainbow trout were most sensitive six times and were never the least sensitive species. Crayfish and snails were generally among the least sensitive species tested. However, crayfish were the most sensitive species with dursban and second only to rainbow trout in sensitivity to sevin. Snails were the second most sensitive to cadmium chloride behind rainbow trout. Fish sensitivity factors (highest fish 96 h LC_{50}/lowest fish 96 h LC_{50}) ranged from 2·1 to 1703 for 2-chloroethanol and cadmium

TABLE 3

The 96 h LC_{50} Values and 95% Confidence Intervals (mg litre^{-1}) for the Various Compounds and Species Tested

Chemical	*Fathead minnow*	*Rainbow trout*	*Goldfish*	*Channel catfish*	*Bluegill*	*Crayfish*	*Snail*	*Fish sensitivity factors*[a]
Pentachlorophenol—Test 1 (species tested individually)	0·223	—[b]	0·117 (0·106–0·128)	<0·053[c]	0·150 (0·127–0·177)	—[b]	0·157 (0·096–0·256)	>4·2
Pentachlorophenol—Test 2 (species tested individually)	0·286 (0·179–0·345)[d]	—[b]	0·156 (0·132–0·184)	0·054 (0·046–0·062)	0·152 (0·127–0·181)	—[b]	—[b]	5·3
Pentachlorophenol—Test 3 (species exposed in separate compartments of the same tank)	0·244 (0·218–0·273)	—[b]	0·191 (0·163–0·255)	0·054 (0·047–0·061)	0·115 (0·103–0·129)	—[b]	0·142 (0·101–0·200)	4·6
2-Chloroethanol	39·5 (35·3–44·2)	35·6 (30·8–41·2)	19·1 (16·8–21·7)	26·4 (23·2–30·1)	21·5 (19·2–24·1)	>80·9[e]	>80·9[e]	2·1
2,4-Pentanedione	155 (111–217)[e]	71·6 (64·5–79·4)	107 (93·5–123)	83·6 (62·8–111)[e]	74·3 (67·9–81·4)	>217[e]	155 (111–217)	2·3
Hexachloroethane	1·23 (1·08–1·40)	0·970 (0·730–1·28)	>2·10[e]	1·52 (1·39–1·65)	0·970 (0·730–1·28)[e]	>2·10[e]	>2·10[e]	>2·2
α-Bromo-2′,5′-dimethoxyacetophenone	0·053 (0·048–0·058)	0·053 (0·048–0·058)	0·158 (0·138–0·182)	0·044 (0·038–0·050)	0·100 (0·082–0·122)	>0·227[e]	>0·227[e]	3·6
Benzaldehyde	12·4 (10·9–14·0)	11·2 (10·6–11·8)	13·8 (10·8–17·6)	5·39 (4·99–5·82)	1·07[f] (0·80–1·44)[e,f]	>15·8[e]	>15·8[e]	12·8
1,3-Dichloro-4,6-dinitrobenzene	0·038 (0·030–0·047)	0·026 (0·024–0·028)	0·047 (0·037–0·059)	0·028 (0·025–0·031)	0·074 (0·044–0·126)[e]	>0·126[e]	>0·126[e]	2·8
Dursban	0·542 (0·225–1·31)	0·009 (0·007–0·011)	>0·806[e]	0·806 (0·434–1·088)[e]	0·010 (0·006–0·014)[e]	0·006 (0·004–0·009)	>0·806[e]	>88·2
Cadmium chloride	1·50 (1·27–1·76)	0·003 (0·002–0·004)	0·748 (0·408–1·37)	4·48 (3·23–6·21)	6·47 (5·60–7·47)	>10·2[e]	0·093 (0·054–0·160)	1 703
Sevin (Carbaryl)	5·01 (4·57–5·48)	0·860 (0·056–2·25)[e]	16·7 (14·8–18·8)	12·4 (12·0–12·8)	6·97 (3·87–10·2)[e]	2·87 (1·55–5·32)	>27·0[e]	19·4

[a] The fish sensitivity factor is highest fish 96 h LC_{50} value divided by the lowest fish 96 h LC_{50} value.
[b] This species was not tested.
[c] Six of ten exposed fish died at 0·053 mg litre^{-1} which was the lowest exposure concentration.
[d] Confidence intervals (>95%) were calculated using the binomial test (Stephan, 1977).
[e] The 96 h LC_{50} was higher than this value which is the highest concentration tested.
[f] This LC_{50} was calculated using data from two separate tests.

chloride (Table 3). Excluding dursban, sevin and cadmium chloride the range is narrowed from 2·1 to 12·8.

Table 4 presents the 24, 48, 72 and 96 h LC_{50} values for all fish species where they were calculable. These data were useful for determining if a species was at or near its mortality threshold for a particular chemical. By threshold it is meant that no more significant mortality is expected if two or more consecutive LC_{50} values do not change significantly.

TABLE 4
The 24, 48, 72 and 96 h LC_{50} Values (mg litre^{-1}) for Ten Tested Chemicals and Five Species of Fish

Compound	*Species*				
	Fathead minnow	*Rainbow trout*	*Goldfish*	*Catfish*	*Bluegill*
Pentachlorophenol No. 1					
24 h	0·023	—	0·120	0·076	0·224
48 h	0·223	—	0·112	0·067	0·201
72 h	0·223	—	0·117	0·059	0·156
96 h	0·223	—	0·117	<0·053	0·150
Pentachlorophenol No. 2					
24 h	0·309	—	0·203	0·085	0·217
48 h	0·286	—	0·203	0·060	0·152
72 h	0·286	—	0·174	0·054	0·152
96 h	0·286	—	0·156	0·054	0·152
Pentachlorophenol No. 3					
24 h	0·244	—	0·228	0·074	0·136
48 h	0·244	—	0·191	0·064	0·121
72 h	0·244	—	0·191	0·058	0·115
96 h	0·244	—	0·191	0·054	0·115
2-Chloroethane					
24 h	—	—	—	—	44·6
48 h	70·2	64·5	29·2	68·8	27·6
72 h	43·5	43·5	23·4	40·9	23·3
96 h	39·5	35·6	19·1	26·4	21·5
2,4-Pentanedione					
24 h	—	101	—	—	155
48 h	186	81·6	155	155	91·7
72 h	155	72·9	133	83·6	77·9
96 h	155	71·6	107	83·6	74·3

(*continued*)

TABLE 4—*contd.*

Compound	*Species*				
	Fathead minnow	*Rainbow trout*	*Goldfish*	*Catfish*	*Bluegill*
Hexachloroethane					
24 h	1·74	1·51	—	—	1·82
48 h	1·44	0·970	—	1·93	1·60
72 h	1·29	0·970	—	1·60	1·13
96 h	1·23	0·970	>2·10	1·52	0·970
α-Bromo-2′,5′-dimethoxyacetophenone					
24 h	0·073	0·058	—	0·080	0·168
48 h	0·058	0·054	0·208	0·051	0·144
72 h	0·054	0·053	0·174	0·050	0·132
96 h	0·053	0·053	0·158	0·044	0·100
Benzaldehyde					
24 h	—	15·8	—	—	5·71
48 h	15·8	12·6	—	10·1	2·59
72 h	13·5	11·4	—	6·16	1·43
96 h	12·4	11·2	13·8	5·39	1·07
1,3-Dichloro-4,6-dinitrobenzene					
24 h	—	0·067	0·086	0·067	0·085
48 h	0·074	0·047	0·067	0·029	0·079
72 h	0·049	0·034	0·051	0·028	0·074
96 h	0·038	0·026	0·047	0·028	0·074
Dursban					
24 h	—	—	—	—	—
48 h	—	—	—	—	—
72 h	—	—	—	0·806	—
96 h	0·542	0·009	>0·806	0·806	0·010
Cadmium					
24 h	1·91	—	—	10·2	—
48 h	1·59	0·004	—	5·02	7·78
72 h	1·50	0·003	—	4·61	7·33
96 h	1·50	0·003	0·748	4·48	6·47
Sevin					
24 h	5·94	0·860	23·8	18·6	10·1
48 h	5·01	0·860	21·4	13·6	9·51
72 h	5·01	0·860	19·3	12·6	8·12
96 h	5·01	0·860	16·7	12·4	6·97

Dimethylformamide (DMF) solvent test

Some chemicals had low water solubility so stock solutions were made using DMF as a solvent carrier. To guarantee that DMF was not killing the organisms, an acute test was conducted with a nominal high concentration of 3680 mg litre^{-1} of DMF. This concentration was 7·7 times higher than the amount used in any other toxicity test (477 mg litre^{-1}) and did not produce any mortality in the highest test concentration with any of the tested species.

DISCUSSION

Conducting acute toxicity tests on several species in different chambers of the same exposure tank presents several distinct advantages. It allows an easy determination of the most sensitive species and cuts cost of labour, materials and chemical analyses for measured concentration tests.

A test with six concentrations that are measured daily usually has eight analytical samples per day, one for each concentration, a duplicate of one concentration and a recovery sample of the control. The total samples per four day, seven species test is 32. If each species were tested individually the total would be 224 analytical samples per toxicant tested. The cost of these analytical samples have been variously estimated at 30–100 US dollars per sample, depending on the instrument used for analysis and amount of sample preparation required. Because all species are treated nearly the same it allows the researcher a side-by-side comparison of the effects of a toxicant on behavioural and physiological parameters that are not possible in separate tests. Narcotic chemicals, for example, may produce clearly defined symptoms in fatheads but entirely different symptoms in catfish, even though they may be physiologically affecting both species the same. Comparing the symptoms in response to a known narcotic enables the researcher to determine if other toxicants are narcotic in their mode of action. The same method of deduction may be used for other modes of action seen for the various toxicants.

On rare occasions dissolved oxygen concentrations fell to as low as 50% saturation. This could alter the LC_{50} somewhat for the chemical being tested, since it places an added stress on the organisms. However, never were the mortalities that were observed different from those that were expected. In addition, all the species were subjected to the same stress since they were in the same exposure chamber.

When the literature is examined for toxicant information, the reader is often confronted with data from a variety of sources and methods on a variety of species of differing sizes and ages. This variability makes it difficult to compare sensitivity differences between several species unless one investigator conducted all the tests using similar methods. Even in this case, there is always some variability between individual tests conducted separately due to several factors. Overall variability in analytical procedures may cause 10 to 15% error in LC_{50} values in a toxicity test. Concentration peaks caused by the dilution apparatus may also be a major factor depending on the toxicant. It is speculated that concentration peaks, rather than the average concentration, cause the mortality observed in some tests. These peaks may be of short duration and not reflected by the measured concentrations. Other variables which may influence LC_{50} values between tests conducted separately are pH, temperature, dissolved oxygen, alkalinity, acidity, turbidity, organic matter, fish health, size and disturbances caused by the researchers to mention a few. Conducting comparative toxicity tests with all fish exposed in separate compartments of individual exposure tanks eliminates this variability that occurs between tests conducted separately.

The present tests were conducted in compartmentalised tanks to keep interspecific interactions to a minimum. It is possible that all tested species could be tested together without partitions. This method could have been verified by conducting tests with all species exposed together in the same tank. However, even if no territoriality or aggressive behaviour were observed, the proof would only apply at that time and at a later time with the same batch of organisms these factors could influence mortality.

It is known that many industrial organic compounds have a non-specific mode of toxic action that causes death by narcosis or general anaesthesia. Chemicals which exhibit this narcotic or anaesthetic mode of action would be expected to have little variation in toxicity between fish species. This appeared to be true with the currently tested organic compounds which had fish sensitivity factors that ranged from 2·1 to 12·8 for 2-chloroethanol and benzaldehyde, respectively. The reason for the high fish sensitivity value for benzaldehyde was the low 96-h LC_{50} value derived for bluegills. It appears there may be a mode of toxic action other than narcosis influencing the toxicity of benzaldehyde to bluegills, although it is unclear what specific organ or enzyme is being affected. With the bluegill 96 h LC_{50} removed, the fish sensitivity factor for benzaldehyde becomes 2·6. Fish sensitivity factors for the two pesticides,

sevin and dursban, and for cadmium chloride ranged from 19·4 to 1703. Species sensitivity differences of this magnitude indicate a need for testing more species with these types of compounds in order to set water quality criteria that will adequately protect important aquatic species.

The present dilution apparatus had a dilution factor of 0·6 and used five exposure concentrations and a control. This dilution factor gives a 7·7-fold difference between the highest and lowest diluter concentrations. Using the present system, species sensitivity differences of more than 7·7 times required conducting additional tests. The greatest number of tests required to determine LC_{50} values for the seven species with a single chemical in the present test series was three (Table 1). However, insufficient mortalities occurred in the high concentration tanks with hexachloroethane versus goldfish, dursban versus goldfish, and in many tests with snails and crayfish to calculate 96-h LC_{50} values for these species. These data were considered adequate to show these species were not among the most sensitive species to these chemicals. These tests were not repeated at higher concentrations and the LC_{50} values are reported (Table 3) as being greater than the highest exposure concentration.

The 96 h LC_{50} value presented for bluegills and benzaldehyde was derived from two tests (Table 4). In the first test all fish had died by 96 h in the lowest concentration (1·44 mg litre^{-1}), while in the second test no fish died in the highest concentration of 0·80 mg litre^{-1}. The 96 h LC_{50} for benzaldehyde and bluegills was calculated on the basis of these values.

Comparability of the present data with single species toxicant tests conducted at this laboratory is good. Fathead minnow 96-h LC_{50} values (μg litre^{-1}) were, in this test, 1230 compared to 1510 (Walbridge *et al.*, 1983), 244 to 225 (Phipps *et al.*, 1981), and 542 to 203 (Holcombe *et al.*, 1982) for hexachloroethane, PCP, and dursban, respectively. Rainbow trout values were 9·14 to 8·0 (Holcombe *et al.*, 1982) for dursban. All values, with the exception of dursban, are within 20 percent, and this variability could be reasonably expected in a repeat test. The fathead minnow LC_{50} value for dursban varied by 73% and this difference may have been caused by size differences of the test fish. Fathead size in the present test was 0·54 g per fish compared to 0·14 g in the earlier test (Holcombe *et al.*, 1982). From these data and data from the three pentachlorophenol tests in the present study, it was concluded that exposing the species together in compartments of the same tanks is a viable and desirable option for conducting comparative toxicity tests.

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